EXPRESSION OF HER-2/NEU IN PATIENTS WITH PRIMARY NON-SMALL CELL LUNG CANCER (NSCLC)

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Abstract:

Introduction: *Now a days immunohistochemistry and genomic testing for patient with Non-Small Cell Lung Cancer (NSCLC) is becoming new standard of care in clinical decision making. A renewed interest has been emerging on the human epidermal growth factor-2 (HER2) pathway. Aim of this present study was clinicopathological correlation of HER-2/neu expression by IHC in NSCLC.*

Method: *This was a Cross sectional and observational study done at Sir Salimullah Medical College and National Institute of Disease of the Chest and Hospital (NIDCH) and other private hospitals in Dhaka city during July / 2014 to June / 2016. Adult of both gender with histologically diagnosed as a case of NSCLC was include in the study. Immunohistochemistry was done to see the positivity for HER/neu and clinical characteristics were observed.*

Result: *A total 45 patients with NSCLC were enrolled in the study. Male was 77.8% (n=35) and female was 22.2% (n=10). The mean age was 55.67 (SD± 12) years and mean age of male was higher compared to female (57.54±11.64 years verses 49.1±11.38 years). Most of the male were smokers (71.1%) and female were nonsmokers (90%). 57.78% (n=26) of patients and 42.22% (n=19) of patients had adenocarcinoma and squamous cell carcinoma respectively. Most squamous cell carcinoma patients were elderly, had wasting and having higher TNM staging. Serum LDH level was higher with advance staging and grading. Only 8.89% (n=4) had HER-2/neu positive expression affected by male. Patients with HER 2/neu positive expression were relatively older (mean age 62.5±17.08). However, there were equal in histopathological categorization (50% SCC and 50% Adenocarcinoma). Mean LDH was slightly higher in HER-2 positive patients was compared to HER-2 negative patients (569.5±232.6 versus 469.7±181.8).*

Conclusion: *About nine percent of patients having HER-2/neu positive was relatively older and had more high level of LDH. A large-scale study should be conducted in Bangladeshi population to characterize epidemiological, clinicopathological feature in patients with NSCLC with HER-2 expression.*

Key wards: *NSCLC, HER-2/neu*

Introduction

Lung cancer has been the most common cancer in the world for several decades. There are estimated to be 1.8 million annually new cases occurred in 2012.[1] Because of high fatality (the overall ratio of mortality to incidence is 0.87) lung cancer is the most common cause of death from cancer worldwide estimated to be responsible for 1.59 million deaths per year (19.4% of total).[1]

For practical purpose, lung cancers are divided into two clinical subgroups – Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC). 80% – 85% of all lung cancers are Non-Small Cell Lung Cancer (NSCLC).[2] Along

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with histomorphology, immunohistochemical and genetical analysis is now used commonly to stratify patient into clinically relevant subgroup.

The therapeutic landscape of Non-Small Cell Lung Cancer (NSCLC) has dramatically changed in the last few years with introduction of molecular targeted agents leading to histological and molecular approach. Demonstrating an overall survival advantage, use of Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitor (TKIs) has emerged as optimal treatment option in selected patient based on activating mutations in tyrosine kinase domain of EGFR.[3, 4]. The discovery of EGFR mutation in NSCLC in 2004 and the marked response to the EGFR TKI, in a small subset of patient harboring these genetic abnormality[5,6] stimulated the study of other kinase mutants involvement in NSCLC.[7]

So far, several members that belong to the EGFR family have been described: HER1 (EGFR/erbB1), HER2 (neu, erbB2), HER3 (erbB3), and HER4 (erbB4)[8]. The human epidermal growth factor receptor (HER) family of receptors plays a central role in the pathogenesis of several human cancers.[8] They regulate cell growth, survival, and differentiation via multiple signal transduction pathways and participate in cellular proliferation and differentiation[9]. All four HER receptors comprise a cysteine-rich extracellular ligand binding site, a transmembrane lipophilic segment, and an intracellular domain with tyrosine kinase catalytic activity.[10] Upon ligands binding to their extracellular domains, HER proteins undergo homo or hetero dimerization results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors initiates a variety of signaling pathways, principally the mitogen-activated protein kinase (MAPK), Phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K), and protein kinase C (PKC) resulting in cell proliferation, survival, differentiation, angiogenesis, and invasion.[7]

Most of the studies on HER2/neu have been carried out in breast cancer, after it was found to induce mammary carcinogenesis.[11] Amplification or overexpression of the HER2

gene occurs in approximately 15–30% of breast cancers.[12] With increasing understanding of HER2/neu biology, it has now been recognized that HER2 overexpression occurs in other forms of cancers also such as colon[13], bladder[14], ovarian[15], uterine endometrial carcinoma[16], stomach[17] and esophagus[18]. The exact percentage of Her2/neu overexpression in NSCLC is difficult to assess since studies reporting overexpression rates ranged from 4% to 27%.[19,20] This heterogeneity is mainly due to differences in the methods used to assess Her2/neu expression.[7] Patients with HER 2 mutant NSCLC have distinct clinicopathological characteristics.[21] In a study of 65 patients with HER 2 mutant NSCLC, median age of diagnosis was 60.4 year (range 31-86), 69% were female, 52% were nonsmoker and all tumours were adinocarcinoma[22].Specialized breast centers recognized that HER2 positive breast tumors to be higher grade and to be estrogen receptor negative, where as well differentiated breast cancer rarely was HER2 positive[23]. In NSCLC correlation of grading or degree of differentiation with HER2 positivity is yet to be established.

A substantial need for energy of tumour cells is by their increased consumption of glucose by the process of glycolysis. Due to the increased glycolysis of tumour tissue not only pyruvate reduction to lactate is accelerated but also the oxidative phosphorylation of pyruvate is diminished.[24,25] The increased production of lactate and H+ ions subsequently reduce the extracellular pH. The acidic environment may increase the ability for invasion[26] and macrophage mediated angiogenesis.[27] This could explain the worse survival of patients with increased serum LDH and it can be a surrogate parameter for molecular marker of prognosis.[24] Recent evidence demonstrates direct link of HER2/neu to glycolysis, and that HER2/neu - overexpressing cells possessed a significantly higher level of glycolysis when compared to the HER2/neu-low-expressing cells.[28,29] It was further shown that NSCLC patients have increased serum LDH.

The relationship between HER 2/ neu and serum LDH level in patients with NSCLC have not been addressed before. So, the present study

was undertaken to observe the expression of HER2/neu in patients with NSCLC and correlate with grading and staging of NSCLC. Furthermore, correlation between expression of HER2/neu and serum level of LDH was also addressed. So far known there was no such previous study in Bangladesh before.

Methods:

This was a Cross sectional and observational study done at Sir Salimullah Medical College and National Institute of Disease of the Chest and Hospital (NIDCH) and other private hospitals in Dhaka city during July / 2014 to June / 2016. Adult of both sex with histologically diagnosed as a case of NSCLC was include in the study. Patients declined for consent and already got radiotherapy or chemotherapy was excluded from the study. Sampling was purposive and convenient sampling and a total number of 45 cases were included in this study.

Collection of specimens for Histopathology:
All the specimens obtained after pneumonectomy or lobectomy operation and were immersed in 10% normal buffered formalin. Samples were fixed for an average 18 hours which was required for proper immunostaining, as underfixation may cause false positive IHC result.

Gross examination: After lobectomy or pneumonectomy sample and section of the blocks were done according to standard surgical pathology. The relevant data were noted in a predesigned data sheet.

Tissue processing and staining: In the department of pathology, NIDCH, Dhaka, the specimens were examined and the gross features regarding number, appearance and measurements were noted in a register book. Then the pieces of tissue were wrapped in tissue paper and placed in a bottle containing 10% formalin. The tissue was then processed according to the routine paraffin embedding method.

Microscopic Analysis: After histopathological examination relevant points were taken from the report and included in the prescribed proforma.

Immuno-Histochemical analysis: Immuno-staining for HER2 was done at AFIP (Armed Forces Institute of Pathology, Dhaka). For immunohistochemistry staining 4-micrometer thick tissue sections were taken on poly-L lysine coated slide from the paraffin blocks of tumor. Primary Antibody: Polyclonal rabbit antihuman cerB-2 oncoprotein (DAKO, code- A0485, Denmark). Secondary Antibody: Envision (ready to use DEKO, code-K5007) was used as secondary antibody. HER2 positive high grade breast carcinoma was considered as Positive control.

Scoring system and cut off value: Tumors with more than 10% of cancer cells showing membranous staining for HER-2 was be classified as positive. Cytoplasmic staining was not considered.

Data processing and analysis: Collected data were checked, edited & then Epi Info 7^{TM} was used for data analyses. Statistical significance was set at p<0.05.

Ethical measures: The study protocol was approved by the ethical committee of Sir Salimullah Medical College, no SSMC/2015/130 Date: 25.10.2015

Observations and Results
A total of 45 patients with NSCLC were enrolled in the study. Tissue was obtained from pneumectomy or lobectomy specimen. After gross examination, haematoxylin and eosin stained section were examined under microscope for histological categorization. Immunohistochemistry was done in all cases for HER 2 /neu expression. Along with histological and immunohistochemical examination clinical parameters, haemoglobin level and preoperative serum LDH level was observed and recorded and correlated.

Demographic Characteristics: In the study age range of the patients were 28 to 85 years. Mean age of the patients was 55.67±12.0 year and median age was 55 year. Among 45 patients, 35(77.8%) patients were male and 10 (22.2%) were female. Male: female ratio was 3.5:1.

Female presented at a significantly younger age (mean age: 49.1±11.38) compared to male (mean

age 57.54±11.65) (P- value - 0.048). However, differences was not significant. All female patients were below 60 years of age. Male patients were predominant in this study.

Clinical characteristics of study subject: Among 45 lung cancer patients, wasting were present in 24(53.3%) of patients. 28(62.22%) patients presented with mild anemia; 10(22.2%) patients presented with moderate anemia. 6 (13.3%) patients presented with clubbing.

Table I

Distribution of lung cancer patients by clinical characteristics (n=45)

Clinical characteristics	Frequency	Percentage (%)
Wasting		
Present	24	53.3
Absent	21	46.7
Anaemia		
No	7	15.6
Mild	28	62.2
Moderate	10	22.2
Severe	0	0
Clubbing		
Present	6	13.3
Absent	39	86.7

Smoking status: Majority of the patients with NSCLC 32 (71.1%) male were smoker and only 1(2.2%) female was smoker.

Histological classification and their characteristics: In the present study 26 (57.78%) patients were diagnosed as adenocarcinoma and 19 (42.22%) patients were diagnosed as squamous cell carcinoma. Among the patients with adenocarcinoma, 69.2% were male and 30.8% were female. Marked male (89.5%) dominance were noted among patients with SCC. Only 2 (10.5%) patients were female having SCC. When compared between two groups, it was found not significant (Table-II)

Proportion of squamous cell carcinoma was increasingly high in elderly age group and conversely younger patients were mostly affected by adenocarcinoma (Table III). Mean age of patients with adenocarcinoma was 49.46±9.06 years and patients with squamous cell carcinoma was 63.58±9.83 years. Most of the patients with adenocarcinoma were in 41-60 years age group and SCC were in 61-80 years age group. Patients with SCC were a bit older compared to patients with adenocarcinoma. In the present study when both groups were compared, it was found statistically significant.

Table-II

Distribution of histological type of lung cancer.

Histopathological Classification	n	Sex		p value
		Male	Female	
Squamous Cell Carcinoma	19	17(89.5%)	2(10.5%)	0.107[ns]
Adenocarcinoma	26	18(69.2%)	8(30.8%)	
Total	45	35(77.8%)	10(22.2%)	

p value reached from Chi-square test, ns = Not significant

Table III

Percent distribution of histological type of lung cancer according to age (n=45)

Age of cancer patients	n	Squamous cell carcinoma No (%)	Adenocarcinoma No. (%)	p value
21-40	5	0.00	5(100%)	
41-60	28	9(32.1%)	19(67.9%)	
61-80	11	9(81.8%)	2(18.2%)	
81-100	1	1(100.0%)	0.00	
Mean±SD	45	63.58±9.83	49.46±9.06	<0.001*

p value reached from Unpaired t-test, *=significant

There were differences of clinical characteristics among histological types. 28 patients with NSCLC were mild anaemic, among them 42.9% were with SCC and 57.1% were with adenocarcinoma, among 10 moderate anaemic patients, 50% were with SCC and 50% were with adenocarcinoma. Wasting was observed in total 24 patients, out of them 54.21% SCC and 45.8% were adenocarcinoma. Clubbing observed in 6 patients, 100% were SCC. Clubbing was significantly more common in squamous cell carcinoma patients (p =0.002). When the clinical characteristics compared between groups, it was found not significant between patients presented with anaemia and wasting but statistically significant when compared patients with clubbing.

Proportion of smoker, was significantly higher in patients with squamous cell carcinoma (94.7%) compared to patients with adenocarcinoma (57.7%). Amount of smoking consumed in pack year was markedly higher in patients with squamous cell carcinoma compared to patients with adenocarcinoma. Mean consumption was in pack year in patients with squamous cell carcinoma was 20.56±5.91 pack year and that of adenocarcinoma was 8.25±4.12 pack year.

Out of 19 SCC patients, 57.9% had stage II and 42.1% patients had stage III. Among 26 adenocarcinoma patients, 19.2% were of stage I, 50.0% were of stage II and 30.8% were of stage III. When compared among different tumor stages it was not statically significant.

Table-IV

Differences of general examination findings in various histological types: anaemia, cyanosis and clubbing

Clinical findings	n	Squamous cell carcinoma No (%)	Adenocarcinoma No. (%)	p value
Anaemia				
No anaemia	7	2(28.6%)	5(71.4%)	
Mild anaemia	28	12(42.9%)	12(57.1%)	0.675^{ns}
Moderate anaemia	10	5(50.0%)	5(50.0%)	
Wasting				
Absent	21	6(28.6%)	15(71.4%)	0.083^{ns}
Present	24	13(54.21%)	11(45.8%)	
Clubbing				
Absent	39	13(33.3%)	26(66.7%)	0.002*
Present	6	6(100.0%)	0(0.0%)	

p value reached from Chi-square test, ns = Not significant

Table-V

Association between tumour staging with histological classification

Tumour staging	Squamous cell carcinoma(n=19) No (%)	Adenocarcinoma (n=26) No. (%)	p value
Stage I	0(0.0%)	5(19.2%)	0.124^{ns}
Stage II	11(57.9%)	13(50.0%)	
Stage III	8(42.1%)	8(30.8%)	
Total19	(100.0%)	26(100.0%)	

p value reached from Chi-square test, ns = Not significant

Regarding LDH, mean LDH level was 480.47±188.05 (U/L) in SCC and 483.08±203.12 (U/L) in adenocarcinoma. There is no significant difference of serum LDH between histological types (SCC vs adenocarcinoma). Mean serum LDH was found gradually increased in advanced tumour stage. Mean serum LDH was 258.80±42.25 (U/L), 475.9±169.8 (U/L) and 551.2±186.9 (U/L) in stage I, stage II and stage III respectively. Differences of mean serum LDH among stages was found statistically significant (p=0.006). HER 2/neu expression: Out of 45 patients, 4(8.89%) were with HER2/neu positive NSCLC revealed by immunohistochemical examination. All patients with HER2/neu positive were male. Mean age of patients with HER2/neu positive (62.50±17.08) was higher compared to patients

with HER/neu negative (54.73±11.05) NSCLC. It is a remarkable that 75% patients with HER2/neu positive expression had presented with wasting and they were smoker also. HER2/neu positive cancers were histopathologically 50% adenocardinoma and 50% squamous cell carcinoma. 75.0% patients with NSCLC were HER2/neu positive in grade II and 25% were in grade III. Regarding TNM staging, 25% of HER2/neu positive was in stage II and 75.0% was in stage III. None of patient, with histological grade-I and having stage I of TNM staging had HER2/neu expression. There was no significant difference mean LDH level in patient with HER2/neu positive and negative expression. LDH level was a bit higher in patients with HER2/neu positive (569.5±232.6) compared to patients with HER2/neu negative expression (469.7±181.8).

Table VI

Compare of serum LDH between SCC and adenocarcinoma patients (n=45)

Variables	Squamous cell carcinoma (n=19) Mean±SD	Adenocarcinoma (n=26) Mean±SD	p value
LDH (U/L)	480.47±188.05	483.08±203.12	0.729ns
Range	(244-928)	(227-886)	

p value reached from Unpaired t-test, ns = Not significant

Table VII

Mean LDH in different staging of tumour

	Tumour staging			P value
	Stage I (n=5) Mean±SD	Stage II (n=24) Mean±SD	Stage III (n=16) Mean±SD	
Serum LDH (U/L)	258.80±42.25	475.9±169.8	551.2±186.9	0.006*

p value reached from ANOVA test, *=significant

Table-VIII

Comparison of HER2/neu positive and negative patients (n =45)

Variables		HER2/neu		p value
		Positive (n=4)	Negagive (n=41)	
Age (in years) (mean±SD)#		62.50±17.08	54.73±11.05	0.207ns
Sex (no. %)##	Male	4(100.0%)	31(75.6%)	0.263ns
	Female	0.0%	10(24.4%)	
Smoking status (no. %)##	Smoker	3(75.0%)	30(73.2%)	0.937ns
	Non smoker	1(25.0%)	11(26.8%)	
Histological classification (no. %)##	SCC	2(50.0%)	17(41.5%)	0.741ns
	Adenocarcinoma	2(50.0%)	10(58.5%)	
Grading (no. %)##	Grade I	0(0.0%)	2(4.9%)	0.005*
	Grade II	3(75.0%)	39(95.1%)	
	Grade III	1(25.0%)	0(0.0%)	
Staging (no. %)##	Stage I	0(0.0%)	5(12.2%)	
	Stage II	1(25.0%)	23(56.1%)	0.215ns
	Stage III	3(75.0%)	13(31.7%)	
Serum LDH (mean±SD)#		569.5±232.6	469.7±181.8	0.311ns

p value reached from #Unpaired t-test and ##Chi-square, *=significant, ns = Not significant

Discussion:

Lung cancer has been the most common cancer in the world for several decade. There are estimated to be 1.8 million new cases in 2012. Lung cancer is also the most common cause of death from cancer worldwide, estimated be responsible for nearly one in five cancer related death. In Bangladesh incidence of lung cancer in male is 16.6 per1000000 and mortality is14.8 per 100000. In female incidence is 3.6 per 100000 and mortality is 3.3 per 100000.[1]

In present study 35 (77.8%) patients with NSCLC were mostly male and 10 (22.2%) patients were female and male to female ratio was 3.5:1 which was higher compared to worldwide male to female ratio of 1.88 :1.[1] However PS Akhter et. al found the male female ratio 6.53:1 when they conducted a study in National Institute of Cancer Research and Hospital on the patients with bronchial carcinoma.[73] Male female ratio in the present study is comparable to that of a study of eastern India.[74] Male predominance reflect higher smoking rate in male (91.43%) compared to female (10%). Female patient usually does not come to physician and does not present to health care facilities in our country.

Age is a risk factor for lung cancer and more than 90% of patients were above 40 years of age at the time of diagnosis in a study.[75] In the present study (Fig:1) age of presentation was below 40 years (11.1%) which is almost similar to (9.06%) described by Bhattacharya et al[76]. Mean age of presentation in this study was 55.67±12.0 years which is lower than age shown by Akhter PS et al[73]. But mean age was similar in studies of eastern India.[74,76]

Female patients with NSCLC presented at a significantly younger age (Fig:3) than male which is comparable to Indian reports[74,76]. All female in this study presented below age of 60 year. Mean age of presentation was 49.1 years in female (SD±11.38) and 57.54 years in male (SD±11.65). Mean age of presentation was much lower compared to a study of eastern India where mean age of presentation in male and female was 54.2 years and 58.9 years respectively.[74] The mean age of incidence of squamous cell carcinoma (Mean-63.58, SD±9.83) is significantly higher compared to adenocarcinoma (Mean-49.50, SD±9.06). Radzikowaska E *et al.* showed that adenocarcinoma and SCLC develop at significantly younger age group compared to SCC in both sex (SCC 62.18 ± 9.03, SCLC 59.85 ± 9.97, adenocarcinoma 59.96 ± 10.54).[77]

Regarding clinical presentation of patients with NSCLC (Table I) clubbing has been reported in 29% of patient with lung cancer and is observed more commonly in NSCLC (35%) compared to SCLC.[78] In this study 13.33% of total patients with NSCLC had clubbing among them 35.9% was squamous cell carcinoma histo-pathologically.

Wasting or cancer cachexia is commonly seen in advanced lung cancer patient and associated with poor prognosis. In this present study wasting was proportionately higher with high TNM staging. 0%, 30% and 80% of patients with NSCLC in the present study were in stage I, stage II and stage III respectively. Kimura et. al showed percentage of the patients with cancer cachexia were 45.6%, 46.1%, 25.5%, 26% in patient having T4, T3, T2 and T1 tumor size respectively[79]. In this present study wasting was more marked which might have compounded by general nutritional status. Patients with squamous cell carcinoma were more frequently wasted and anaemic compared to adenocarcinoma (wasting-68.18% versus 38.5%; anemia - 89.47% versus 59.96%). Anaemia was progressively higher with higher TNM stage in the present study. Various factors like older age of presentation, high smoking consumption, advanced TNM staging may have contributed for wasting and anemia in patients with squamous cell carcinoma in the present study.

There are overwhelming evidence that the smoking causes lung carcinoma.[80] There are two categories of evidence that indicate smoking to be the major cause of human lung cancer. Without exception, epidemiological studies have demonstrated a consistent association between smoking and lung cancerciation in women.[81,82] Chemical analyses of cigarette smoke reveal a multitude of known mutagens and carcinogens.[80] Moreover, these chemicals are

absorbed, are metabolized, and cause demonstrable genetic changes in smokers[80].In this present study 71.1% were smoker (Fig. 4). Proportion of patients having habit of smoking is lower in the present study compared to 91.3 % shown by PS Akhter[73] but higher compared to 67.2% shown by Dey A.[74] The quantity of consumption of smoking was also high among the smoker. Mean consumption was 14.76 pack year. 94.7% patients with squamous cell carcinoma were smoker. Only single female patient in the present study was nonsmoker and diagnosed as squamous cell cancer. Approximately 10-15% of cases of lung carcinoma occur in nonsmoker.[77] In this present study only 10% of female with NSCLC were smoker and all female patients with adenocarcinoma were nonsmoker. It has been suggested that gender specific susceptibility of lung cancer,[82] male sex usually associated with Squamous cell carcinoma and female sex usually associated with adenocarcinoma.[77]

Comparing the smoking habit between histological types, not only percentage of smoking was higher patients with squamous cell carcinoma compared to adenocarcinoma (94.74% versus 57.7%) but also quantity of consumption was higher in squamous cell carcinoma compared to adenocarcinoma (20 pack year versus 8.25 pack year). Radzikowska et al. concluded that the association of smoking and lung cancer is most strong among SCC and SCLC, whereas it is weaker for adenocarcinoma.[77] There exists a dose-response relationship between smoking and lung cancer which emphasizes the importance of duration and intensity of smoking as well as number of cigarettes smoked.[77] Compared to never-smokers, smokers have 20-fold increased risk of lung cancer at present.[83] The risk of lung cancer among smokers increase with duration of smoking and number of cigarettes per day.[83] The risk for lung cancer in former smoker remains high than never-smoker even after >40 years of abstinence.[83] In the present study mean quantity of smoking consumption 14.76±8.03 pack year (Fig.5).

In this present study mean LDH level was 481.98±194.71(U/L) in patients with NSCLC. It is higher compared to mean LDH level 406.65(U/L) in patients with NSCLC by Ziaian B et al.[24] There was modest difference of level of serum LDH between histological types in the present study. Mean LDH level is a bit higher in patients with adenocarcinoma group (483.08 U/L) compared to squamous cell carcinoma (480.47 U/L) group. Giroux Leprieur E et. al showed high serum LDH association with advanced stage and poor prognosis.[84] In the present study there was a tendency of increasing level of serum LDH level higher TNM staging. Mean LDH level was highest in stage III (538.20), higher in stage II(484.60) than stage I(243.00) and it was found statistically significant (P value 0.006).

In this present study frequency of HER2/neu positivity was 8.89%. HER2 overexpression has been reported with different frequency values in NSCLC patients with extremely wide ranges.[19,20] Frequency disparities are likely due to differences in the methodologies applied and patient populations studied[7]. Immuno-histochemistry (IHC) was the most frequently used method to detect HER2 overexpression. However, immuno- histochemical results can vary according to the primary anti- body used, the dilution of the antibody, the different tissue samples used (i.e., paraffin-embedded versus frozen samples), the cutoff used for establishing HER2 positivity and whether membrane or cytoplasmic reactivity are assessed[7]. In addition, different patient populations were evaluated, creating possible interpretation bias because low number of patients in some studies[24] might explain the inconsistency of the prognostic impact of HER2 overexpression.[7] Moreover, the differences in the histology of tumors and the stage might have had an impact in these discrepancies. Restricting the frequency analysis to the studies[19] utilizing the IHC Hercep Test, the overall frequency of HER2 overexpression, ranges from 4.3%[85,86] to 34.9%[87], reaching higher frequencies in selected patients' populations, such as bronchioloalveolar carcinoma patients (43%).[88]

Mean age of patients with positive HER2 is higher compared to HER 2 negatives (62.50±17.08 versus 54.73±11.05, p-value 0.207) in the present study (Table-IX). In large

European cohort (EUHER2 cohort)[89] mean age of HER 2 positive patients were 61 years and percentage of male and female were 37.6% male 62.4% respectively, mostly affected by never smokers (60.4%). In this study all patients with HER2 positive NSCLC were male and 75% were smoker and this finding does not match with finding EUHER2 cohort report which could be due to small sample size. IHC positivity has been variously associated with different clinic-pathological features, including adeno-carcinoma histology, advanced metastatic disease, nodal involvement, poor tumor differentiation, lower performance status, and weight loss at baseline[7]. In the present study adenocarcinoma and squamous cell carcinoma equal in total Her2 positive cancer. Out of 4 HER 2/neu positive cases, 75% (n=3) were with grade II and 25%(n=1) case were with grade III cancer.

Ziaian B, et al. showed association of pleural LDH level and plasma LDH level with HER 2/neu expression.[24] Pleural fluid analysis was not done in this study. Serum LDL was measured in this study and mean serum level of LDH was modestly higher in HER 2/neu positive patient compared to HER 2/neu negative patient (569.5 ±232.6 versus 469.7±181.8, P-value-0.311). However, comparison was not statistically significant.

Not only sample size of the study was inadequate for limitation of time but also the number of HER 2/neu positive cases was found limited. So no significant conclusion could be drown between clinicopathological pattern and association with HER 2/neu expression. For the first time HER2/neu expression was demonstrated in NSCLC in Bangladesh on a small sample size. Therefore, it warranted further research with larger sample to evaluate epidemiological, clinical-pathological pattern present in HER 2/neu expression. Genetic mutation is also associated with HER2/neu over expression, mutation should also be evaluated with FISH along with immunohistochemical expression.

Conclusion

Immunohistochemistry for HER-2 expression, serum LDH should be done in NSCLC patients as prognostic biomarker and therapeutic guidance. A large-scale study should be conducted in Bangladeshi population to characterize epidemiological, clinicopathological feature in patients with NSCLC with HER-2 expression. Molecular testing such as FISH (Fluorescence In Situ Hybridiazation) should be used to detect mutation associated with HER 2 overexpression.

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